Bayer HealthCare
Bayer Schering Pharma

Department: GDD-GED Toxicology

☐ Research Report
☐ Product Report
☐ Development-Product Report
☐ Methods Report
☒ Toxicology Report

Report No.: AT06078

Test Item: **PES Vorstufe 2342**

Title: **Acute toxicity in the rat after oral administration**

Study No.: T 4081834

Author(s): U. Gillissen

Study Completion Date: October 14, 2010

Performing Laboratory:

Bayer Schering Pharma AG
GDD-GED Toxicology
42096 Wuppertal
Germany

Sponsor:

Bayer MaterialScience AG

51368 Leverkusen
Germany

GLP Compliance Statement

This study was conducted in compliance with the OECD Principles of Good Laboratory Practice as revised in 1997 (ENV/MC/CHEM(98)17) and with the revised German Principles of Good Laboratory Practice according to Annex I German Chemicals Act (Bundesgesetzblatt, Volume 2008, Part I, No 28, 1173-1184, issued July 11, 2008):

U. Gillissen — Oct. 9, 2010

U. Gillissen
Study Director

Date

Quality Assurance Statement

Study No.: T4081834

Test Item: PES Vorstufe 2342

On the dates given below inspections were conducted by the Quality Assurance to ensure that no deviations exist that are likely to affect the integrity of this study.
The Quality Assurance Unit monitors the conduct of each study by study-based inspections or by process-based inspections of a similar type of study if the short-term nature of a study precludes inspection while it is in progress. Routine procedures and the equipment used in the relevant laboratory areas are inspected regularly and reports are made in accordance with current SOPs.

*(study plan amendments, if any, were duly audited and reported to the Study Director and Management)

Date of Audits / Inspections	**Phases Audited / Inspected**		**Date of Report to Study Director and Management**
Aug-13-2010	Study Plan *		Aug-13-2010
Aug-18-2010	process based	Administration / Dosing, Clinical Observation, Raw Data / Documentation, Preparation of Formulation, Weighing	Aug-18-2010
Oct-01-2010	Main Report	1. Draft	Oct-01-2010
Oct-08-2010	Main Report	Final Draft	Oct-08-2010

The results of this study including the methods used have been checked on the basis of the current SOPs.
They have been correctly reported and the report reflects the raw data.
In case of a multi-site study audits at the test sites are presented in the QA Statement of the Principal Investigator's report (see appendix).

Quality Assurance Unit
Global R&D Quality, GLP-Mgmt.

Date: Oct-08-2010

Signature: 

Christina Kiedrowski

Signatures

Study Director October 14, 2010 U. Gillissen
Date (U. Gillissen)

Test Facility Management October 14, 2010
Date (Dr. C. Stark)

Table of Contents

List of Abbreviations

In addition to the abbreviations for the basic units of the International Unit System (SI) stipulated by law, and designations for decimal multiples and parts of units, the following abbreviations are used:

'	minute
%	percent
a.m.	(ante meridiem) = before noon
approx.	approximately
bw	body weight
C.A.	Chemical Abstracts
CAS	Chemical Abstracts Service
d	day
E	(Endsektion) = final necropsy
e.g.	(exempli gratia) = for example
ff	following
GHS	Globally Harmonized Classification System
h	hour
i.e.	(id est) = that is
LD50	median lethal dose
m	mean
M	moribund sacrifice
max. intens.	maximum intensity
n.a.o.	no abnormality observed
no.	number
p.m	(post meridiem) = after noon
p.o./PO	(per os) = oral
RSD	relative standard deviation
SD/s	standard deviation
T	(Tod) = death
time of d.	time of death

1. Summary

This study was performed to assess the acute oral toxicity to Wistar rats of PES Vorstufe 2342 (content: 100%).

The test item was formulated in polyethylene glycol 400; the administration volume was 10 ml/kg body weight.

The results are summarized in the table below.

Table 1-1 Dose-Response

dose mg/kg bw	toxicological result*	occurrence of signs	time of death	mortality (%)
female				
(1st) 2000	0 / 0 / 3	--	--	0
(2nd) 2000	0 / 0 / 3	--	--	0

* number of animals which died spontaneously and/or were sacrificed in moribund state / number of animals with signs of toxicity / total number of animals used per group

According to OECD guideline 423 the LD50 oral of PES Vorstufe 2342 is >2000 mg/kg bw- equivalent to Category 5 / unclassified of the GHS (>2000-5000) and LD 50 cut off >5000 mg/kg bw according to OECD Test Guideline 423. So it is regarded as non-toxic after oral application.

A dose of 2000 mg/kg body weight was tolerated by female rats without mortalities, clinical signs, effects on weight gain and gross pathological findings.

2. Introduction

The study objective was to determine acute oral toxicity. Information derived from this test serves to indicate the possible existence of hazards likely to arise from short-term exposure by the oral route of the test item, and - with respect to a proper handling and use - serves to permit classification (labeling) of a product.

3. General Information

The study was sponsored by Bayer MaterialScience AG,
51368 Leverkusen, Germany.

The study was performed at Bayer Schering Pharma AG, GDD-GED General Toxicology, 42096 Wuppertal, Germany.

3.1 Responsibilities

Study Director	U. Gillissen
Test Facility Management	Dr. C. Stark
Head of Test Facility	Dr. F.-W. Jekat
Analytic of Formulations	M. Garcia-Sanchez
Archiving	R. Zils
Head of Quality Assurance Unit	Dr. A. Paeßens

3.2 Key Study Data

Study No.	T 4081834
Study initiation date	2010-08-12 (YYYY-MM-DD)
Experimental starting date	2010-08-18 (YYYY-MM-DD)
Experimental completion date	2010-09-08 (YYYY-MM-DD)
Study completion date	see signature page

3.3 Archiving

The study protocol, raw data and final report are retained in the archives specified by the test facility Toxicology of the Bayer Schering Pharma AG in Wuppertal. A retention sample of the test item, and, if applicable, also of the reference item is stored in the archive of the test facility.

4. Material and Methods

4.1 Guidelines

The method used complied with the OECD - Guideline for Testing of Chemicals No. 423 -"Acute Oral Toxicity - Acute Toxic Class Method"; adopted: 17th December 2001; EEC Directive 440/2008 Part B – Method B.1.tris, test methods pursuant to Regulation (EC) No 1907/2006 (REACH), EU Directive 67/548/EWG and EG Regulation 1272/2008.

4.2 Test Item

Test item:	PES Vorstufe 2342
Synonym(s):	Ester Rizinus + Sojaoel-Umesterung
EC No.:	919-697-6
Chemical name:	Castor Oil, reaction product with Soybean Oil
Batch no.:	LB06603520
Appearance:	light yellow liquid
Content of test item*:	100 %
Storage*:	refrigerator
Expiry date:	2010-10-22

*due to product information given by the sponsor

4.3 Test Item Formulation and Analytical Examination in Vehicle

4.3.1 Formulation of Test Item

For oral administration (gavage), PES Vorstufe 2342 was formulated in polyethylene glycol 400. The applied formulations were well mixed before administration. The formulations for administration were prepared at room temperature.

4.3.2 Administration of the Formulation

The dosing is based on the test item. The individual administration volumes were calculated on the base of the body weight at time of administration and were administered in a single oral administration by gavage. The administration volume was 10 ml/kg body weight.

For administration, food was withheld from the animals for approximately 16 - 24 h before administration of the test item, and they were fed again approximately 2 - 4 h after administration.

4.3.3 Analysis of the Formulation

The analysis on stability was performed and archived under study no. 2010/0087/19 (Currenta GmbH&Co.OHG, Services Analytic, Leverkusen)

Table 4-1 Stability in the administration vehicle

nominal value in mg/ml	theoretical value mg/ml	absorbance near 1737 cm^{-1} (mean value) mg/ml		recovery as % of start value	
		start	after 2 hours	start	after 2 hours
2	1.966	0.05994	0.06510	100	109
200	199.9	0.17250	0.16556	100	96

Table 4-2 Homogeneity in the administration vehicle

High concentration	200	mg/ml
The formulation was turbid		
absorbance near 1737 cm^{-1}		
immediately measured		
Upper phase	0.17543	
Middle phase	0.16956	
Lower phase	0.17825	
Relative standard deviation	2.5 %	

The analytical data verify that the test item formulations are stable at room temperature for at least 2 hours and that the test item is homogeneously distributed in the 200 mg/ml formulations. Suspensions had to be stirred for homogenization, the low concentration (2 mg/ml) was clear.

4.4 Number of Animals and Dose Levels

Three animals were used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.:

- no further testing is needed,
- dosing of three additional animals, with the same dose,
- dosing of three additional animals at the next higher or the next lower dose level.

The test item was tested using a stepwise procedure, each step using three animals of a single sex (normally females). The procedure is described in the flow charts of Annex 2, OECD guideline 423.

4.5 Experimental Animals and Housing Conditions

The study was performed in female Wistar rats. The used strain was HsdCpb:Wu (breeder: Harlan GmbH, 5960 AD Horst, Netherlands). Animals of this strain have been used at Bayer Schering Pharma AG for toxicological studies for many years. Historical data on their physiology and spontaneous alterations are available. The state of health of the breeding colony is routinely spot-checked for the main specific pathogens. The results of these examinations are archived.

At start of the study the animals were nulliparous and non-pregnant and free of all clinical symptoms or diseases. The acclimatization time in the animal room was at least 5 days.

Body weights at start of study:
169 g - 204 g

This is according to an age of 8 - 12 weeks approximately.

The animals were assigned to their groups by randomization. The random list was based on evenly distributed chance numbers by a software application. The animals were identified by labels on the cages stating study number, test item, animal number, group number, etc. and by individual animal identification using permanent skin marking.

4.5.1 Husbandry and Nutrition

The animal room had a standardized climate:

Room temperature	22 ±2°C
Air humidity	55 ±5%
Ventilation	approx. 10 changes per hour

Light/Dark cycle 12 hours rhythm.

Occasional deviations from these standards occurred, e.g. during cleaning of the animal room. They did not have any apparent influence on the outcome of the study. The animal room was provided with sound from a radio program.

The animals were group caged conventionally in polycarbonate cages on low dust wood granulate bedding (Lignocel BK 8-15, Firma Rettenmaier, Germany). The cages of the animals were placed on racks. The wood granulate was randomly checked for contaminants at regular intervals and the results have been stored at the Department for Laboratory Animal Services, Bayer Schering Pharma AG, 42096 Wuppertal, Germany. The analyses yielded no evidence of any adverse effects on the aim of the study. Wooden blocks for environmental enrichment were added to each cage. As soon as necessary, they were replaced by new ones. The cages were changed at least once a week. Feed racks and water bottles were not changed. All cage material was washed with hot water. In the first stage of the washing programs an alkaline cleaning agent (Neodisher Alka 300; Chemische Fabrik Dr. Weigert GmbH & Co. KG, concentration: 2.2 g/l) was used.

The animals received the standard diet "Provimi Kliba 3883 PM S15 Maus/Ratte Haltung, Kaiseraugst Switzerland", and tap water ad libitum from polycarbonate bottles.

The nutritive composition and the contaminant content of the standard diet were checked and analyzed routinely in random samples. Nothing untoward was found. The tap water was of drinking water quality (according to the Drinking Water Decree in the current version). The results of the analyses have been stored at Bayer Schering Pharma AG, 42096 Wuppertal, Germany. The available data yielded no evidence of any adverse effects on the aim of the study. The food was available from racks in the lid of the cage, polycarbonate bottles were used for drinking water.

The animal room was cleaned and disinfected weekly. A continuous pest control was performed using a cockroach trap without pesticides (e.g. Killgerm Roach Trap, Killgerm GmbH, 41460 Neuss, Germany). The contact of the animals with the traps was avoided in any case.

4.6 Observations

Clinical signs and mortality rates were determined several times on the day of administration and subsequently at least once daily for an observation period of at least 14 days. Mortality and in the event of symptoms occurring, nature, duration and intensity (possible grading: no intensity specified / 1 = slight / 2 = distinct) were recorded individually. The day of administration is defined as day 1. Times after administration until the following day were recorded either in minutes or in hours, depending on what was appropriate. According to international agreements minutes are given in 5-minute intervals (0' – 2.4' is given as 0', 2.5' – 7.4'is given as 5' and so on). Hours were rounded to full hours. In contrast to this, all further observation intervals are given in days. The duration of the symptoms and the times of death are given relative to the time of administration to the individual animal. The real time points can be taken from the raw data. In general, death was taken as a symptom. Due to the computer system used, death is not shown as a clinical symptom in the lists of the appendix. If no symptoms were seen until death, time of death was taken as the first occurrence of a symptom. In the results section, the findings are summarized without any indication of intensity. The findings can be found for the groups and individual animals in the appendix.

The weight gain of the animals was checked weekly until the end of the study. The weights are given in the tables in the appendix as individual and mean values. The weight gain of the animals was calculated based on rounded individual values. The weights are given in grams (g). Indicated under the heading day 8 are e.g. the data obtained on the 7th day after administration.

Animals which died or were killed in moribund state were weighed (except on day of administration) and dissected as soon as possible, and examined macroscopically. The surviving animals were sacrificed by carbon dioxide at the end of the study, dissected and examined macroscopically.

In some lists of the appendix, data of the same dose are shown as one group, although two subgroups were used.

4.7 Collection, Processing and Evaluation of Data

During this study for collection, storage and evaluation of data a validated LAN-linked computer system was used, which is designed and created in-house. If necessary the data were collected offline.

Hardware and operating systems:

- HP-1000 A (4x, operating system RTE-A 6.2)
- HP-3000 series 900 (1x, operating system MPE/iX 5.5)
- HP-9000 series 200 (1x, operating system HP-UX 11.0)

Software: Data was stored on HP-3000 in an HP TurboImmage/XL Database.

4.8 Calculation of the LD50

The LD50 value was estimated according to OECD - Guideline for Testing of Chemicals No. 423 -"Acute Oral Toxicity - Acute Toxic Class Method"; adopted: December 17, 2001.

5. Results

5.1 Dose-Response Table (LD50)

The results of the study for acute oral toxicity in the fasted rat, including the LD50, are summarized in the tables below.

Table 5-1 Dose-Response

dose mg/kg bw	toxicological result*	occurrence of signs	time of death	mortality (%)
female				
(1st) 2000	0 / 0 / 3	--	--	0
(2nd) 2000	0 / 0 / 3	--	--	0

* number of animals which died spontaneously and/or were sacrificed in moribund state / number of animals with signs of toxicity / total number of animals used per group

LD50 oral: >2000 mg/kg bw- equivalent to Category 5 / unclassified of the GHS (>2000-5000) and LD 50 cut off >5000 mg/kg bw according to OECD Test Guideline 423

5.2 Clinical Signs

No clinical signs were observed (see appendix page 23 and 24).

5.3 Body Weights

There were no toxicologically significant effects on body weight or body weight gain.

The body weights are given in the appendix as individual and mean values on page 25. The body weight gain is given on page 26.

5.4 Gross Pathology Findings

The necropsies performed at the end of the study revealed no particular findings (see appendix, page 27).

6. Conclusion

According to OECD guideline 423 the LD50 oral of PES Vorstufe 2342 is >2000 mg/kg bw- equivalent to Category 5 / unclassified of the GHS (>2000-5000) and LD 50 cut off >5000 mg/kg bw according to OECD Test Guideline 423. So it is regarded as non-toxic after oral application.

Ministerium für Arbeit, Gesundheit und Soziales
Des Landes Nordrhein-Westfalen

Fürstenwall 25, 40219 Düsseldorf Aktenzeichen II A 5 – 31.11.46.06

Gute Laborpraxis/Good Laboratory Practice
GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

Bayer HealthCare AG
BSP-GDD-GED
Toxikologie
Aprather Weg 18 a
42096 Wuppertal

Prüfungen nach Kategorien (gemäß ChemVwV-GLP Nr. 5.3/OECD guidance)	Areas of Expertise (according ChemVwV GLP Nr. 5.3/OECD guidance)
Kategorie 1 Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen	**category 1** physical-chemical testing
Kategorie 2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften	**category 2** toxicity studies
Kategorie 3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)	**category 3** mutagenicity studies
Kategorie 9 Biochemische Toxikologie; Kurzzeitkanzerogenese; Immuntoxikologie; Sicherheitspharmakologie	**category 9** biochemical toxikology; short time cancerogenicity; immunotoxicity; safety pharmacology
Datum der Inspektion 01.Sept.2008 bis 05.Sept.2008	**Date of Inspection** September 1st 2008 until September 5th 2008

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Düsseldorf, den 09.02.2009
Im Auftrag

(Dr. Deden)

The above mentioned test facility/ test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Land Nordrhein-Westfalen 13 Ministerium für Arbeit, Gesundheit und Soziales

Dienstsiegel/official-seal

T4081834 clinical signs; groups Akut/acute ID13235/10

clinical signs	incidence	duration of signs	max. intens.	time of death
2000 MG/KG female PO				
n.a.o.				

T4081834 clinical signs; individual Akut/acute ID13225/10

animal no.	clinical signs	duration of signs	max. intens.	time of death
	2000 MG/KG female PO			
1	n.a.o.			
2	n.a.o.			
3	n.a.o.			
4	n.a.o.			
5	n.a.o.			
6	n.a.o.			

T4081834 Tiergewichte / body weights (G) Akut/acute 13205/10

Tiernr./ animalno.	Tag / day 1	8	15	nach Tod after death	Todeszeit time of d.
2000 MG/KG weiblich / female PO					
1	173	185	206		
2	169	194	210		
3	176	209	221		
4	204	234	246		
5	191	215	226		
6	179	207	218		
m	182	207	221		
s	13.1	17.0	14.2		

T4081834 Gewichtsentwicklung / weight gain (G) Akut/acute 13215/10

Tiernr./ animalno.	Tag / day 1	8	15	Gesamtgew.-Entw./ total weight gain
	2000 MG/KG	weiblich / female	PO	
1	173	+12	+21	+33
2	169	+25	+16	+41
3	176	+33	+12	+45
4	204	+30	+12	+42
5	191	+24	+11	+35
6	179	+28	+11	+39
m	182	25	14	39
s	13.1	7.3	4.0	4.5

Individual macroscopic findings

All findings

animal no.	time / type of death	finding
group 01 2000 MG/KG female PO		
1	15d / E	General observations
		no pathological finding
2	15d / E	General observations
		no pathological finding
3	15d / E	General observations
		no pathological finding
group 02 2000 MG/KG female PO		
4	15d / E	General observations
		no pathological finding
5	15d / E	General observations
		no pathological finding
6	15d / E	General observations
		no pathological finding

Bayer HealthCare
Bayer Schering Pharma

Report No.: AT06078A

PES Vorstufe 2342

1st Amendment to AT06078 of October 14, 2010

Acute toxicity in the rat
after oral administration

DATA REQUIREMENT
Guidelines: Regulation (EC) No 1907/2006 (Reach)
EEC Directive 440/2008 Part B – Method B.1.tris
OECD 423 (2001)
EPA Health Effects Test Guidelines (OPPTS 870.1100)

Report of Study: T 4081834
by
U. Gillissen

Performing Laboratory:

Bayer Schering Pharma AG
GDD-GED Toxicology
42096 Wuppertal
Germany

Sponsor:

Bayer MaterialScience AG

51368 Leverkusen
Germany

Study Completion Date: October 14, 2010
Report Amendment Completion Date: November 02, 2010

GLP Compliance Statement

This amendment was prepared in compliance with the OECD Principles of Good Laboratory Practice as revised in 1997 (ENV/MC/CHEM(98)17) and with the revised German Principles of Good Laboratory Practice according to Annex I German Chemicals Act (Bundesgesetzblatt, Volume 2008, Part I, No 28, 1173-1184, issued July 11, 2008):

U. Gillissen — Oct. 27, 2010

U. Gillissen
Study Director

Date

Table of Contents

Quality Assurance Statement

Study No.: T 4081834
Test Item: PES Vorstufe 2342

This amendment has been reviewed by the Quality Assurance Unit in compliance with the Good Laboratory Practice regulations. On the dates given below inspections were conducted by the Quality Assurance to ensure that no deviations exist that are likely to affect the integrity of this study.
The data presented in this amendment have been reported correctly.

Date of Audits / Inspections	Phases Audited / Inspected	Date of Report to Study Director and Management
Oct-29-2010	Amendment to report 1. Draft	Oct-29-2010
Oct-29-2010	Amendment to report Final Draft	Oct-29-2010

Quality Assurance Unit
Global R&D Quality, GLP-Mgmt.

Date: Oct-29-2010

Signature: 
Ch. Kiedrowski

1. Signatures

Study Director: November 2, 2010 U. Gillissen
Date (U. Gillissen)

Test Facility
Management: November 2, 2010 C. Stark
Date (Dr. C. Stark)

2. Reason for the Amendment

In summary, dose response table and conclusion the classification was not correctly reflected.

3. Correction

The correct classification is:

According to OECD guideline 423 the LD50 oral of PES Vorstufe2342 is > 5000 mg/kg bw. According to EU Directive 67/548/EWG and EG Regulation 1272/2008 the test item is unclassified. So it is regarded as non-toxic after oral application